

EFFICACY OF A SODIUM PERBORATE AGENT FOR PROPHYLACTIC DISINFECTION OF WATERFOWL INCUBATORS

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Summary

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The results of two examinations on the effect of a sodium perborate bleaching agent (Oksisept) for cleaning and disinfection of waterfowl incubators, applied by coarse spray and cold aerosol spray are presented. The comparative evaluation of the disinfection was done via microbiological examinations of the microbial count reduction, presence of residual microflora, and detection of indicator bacterial species. The efficacy of both disinfection protocols was assessed as high. The elements of the ventilation/cooling system of the incubator were evaluated as critical for disinfection, due to the high microbial contamination rate and the more difficult cleaning and disinfection.

Key words: disinfection, hatchery, Oksisept

INTRODUCTION

Disinfection of hatcheries is an essential element of biosecurity measures. Investigations on microbial contamination rates in the different parts of hatcheries showed highest microbial loads in the incubator sector (Ayubi, 1994). Ayubi *et al.* (1996) reported a rapid and marked increase in microbial contamination rate at the beginning of mass hatching.

According to the production technology, the incubator presents the highest risk for infection of hatchlings. There is a probability for incubation of eggs, contaminated either primarily or secondarily with microbial pathogens and when cleaning and disinfection are not properly performed, the infectious agents could be consecutively spread from one batch of hatchlings to another (Ayubi, 1994). That

is why, disinfection is believed to be a primary way to reduce epidemiologic risks.

During the last years, the requirements to disinfection preparations became substantially higher, especially with regard to their ecological effect and human health impact. That is why, new preparations with improved features are constantly implemented. For example, disinfectants from the group of oxidants containing sodium perborate and tetraacetylene-diamine possess a high spectrum of activity and are environment-friendly (Russel & Russel, 1995; McDonnel & Russell, 1999). Therefore, the studies on their efficacy in industrial hatcheries are of special interest.

The purpose of this research was to evaluate the effect of disinfection of a bleaching disinfecting agent applied by coarse spray and cold aerosol spray (cold fog) under the specific environmental conditions of a duck incubator.

MATERIALS AND METHODS

The investigations were carried out in one of currently operating duck hatcheries. Two disinfections of Danno Serie LM (Loudeac, France) incubators (size 2.9×2.9×2.2 m) were carried out.

After removing hatchlings from incubators, surfaces were mechanically cleaned and washed with water under high pressure using a waterblaster, until sensory cleanliness (lack of visible dirt and easy identification of surface traits such as structure and colour) was achieved.

The experiment was carried out with the bleaching agent Oksisept (Zhivas, Sofia, Bulgaria) containing 50% sodium perborate monohydrate and 25% tetraacetylenediamine.

Disinfection treatments were performed after drying of all surfaces and hermetisation of incubators. Working disinfectant solutions were prepared 30 min before application in order to being activated, according to manufacturer's instructions.

Disinfection 1 was conducted by spraying of 2% aqueous solution of Oksisept at a flow rate of 0.5 L/m², using an electric sprayer. The total area for disinfection was 43 m². The ambient temperature during the disinfection was 30°C, and air relative humidity – 85%. The time of exposure was 30 min.

Disinfection 2 was performed by application of 5% aqueous Oksisept solution as a cold aerosol spray (cold fog) with particle size 20–30 µm using a Micro-Jet

ULV 7401 fogmaster. The preparation was sprayed at 30 mL/m³ after preliminary hermetisation of the incubator. The total disinfection volume was 18.5 m³. The ambient temperature during the disinfection was 30 °C, and air relative humidity – 85%. The time of exposure was 30 min.

The microbiological control on the effect of performed disinfections was done through the following methods: achieved microbial count reduction, establishment of residual microflora, and detection of indicator bacterial species presence (Iliev *et al.*, 1982; Arsov *et al.*, 1988; Ekizoglu *et al.*, 2003; Urban *et al.*, 2003; Karadzhov *et al.*, 2004; Slavchev *et al.*, 2005; Angelov *et al.* 2006; Baychev & Karadzhov, 2006). The presence and extent of microbial contamination were assessed on the following surfaces of incubators: incubator wall (including joints), floor (including angles), ventilation/heating system (propeller, heaters etc.)

Surface samples were collected after the incubators were freed from hatchlings, after the mechanical surface cleaning and after the disinfection.

Ten swab samples were also rubbed against each surface for detection of coliforms. Inoculations were made on selective MacConkey Agar (Merck) using routine techniques for identification of coliforms.

From each surface, 3 swabs were obtained for quantitative determination of the total number of mesophilic aerobes and facultative anaerobes. Surface samples were obtained by the microbiological swab method by rubbing sterile swabs against a surface of 20 cm² outlined by a sterile metal template. Swab samples were placed in tubes with 2 mL sterile saline. At the laboratory, tubes were filled up to 10 mL with sterile physiological saline (basic dilution). After multiple washings,

the swab was removed from the tube, and the washing liquid was used to prepare decimal dilutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5}).

Inoculations were made on soybean casein agar (CASO Agar, Merck) to determine the total viable microbial counts on 1 cm² (CFU/cm²). The average count per cm² was calculated from the results of the three swabs for each surface.

For evaluation of the disinfection effect, percentages of microbial reduction representing the difference of surface microbial loads (CFU/cm²) before and after the treatment were calculated (Karadzhev *et al.*, 2004; Slavchev *et al.*, 2005). Log reduction rates were calculated by the following formula (Ekizoglu *et al.*, 2003): $\text{Log}_{10} \text{reduction} = \text{Log}_{10}(\text{CFU/cm}^2 \text{ prior to disinfection}) - \text{Log}_{10}(\text{CFU/cm}^2 \text{ after disinfection})$.

The statistical analysis was done with the Stat Most software.

RESULTS

Microbiological tests in incubators after their liberation from hatchlings (Tables 1, 2) revealed a high extent of microbial contamination – 8.19×10^6 CFU/cm² in average. The contamination rates of the different surfaces of incubators varied, with highest contamination rate of floors (in average, 1.58×10^7 CFU/cm²), followed by ventilation system elements (8.06×10^6 CFU/cm²). The lowest microbial load was observed on the walls of incubators – 6.8×10^5 CFU/cm² in average.

After mechanical cleaning and washing with water under high pressure, the microbial contamination was substantially reduced: the average amount of total viable microorganisms on tested surfaces was 1.69×10^5 CFU/cm². The percentage of microbial reduction after the mechanical cleaning was 97.94% (average for both disinfections).

Table 1. Total number of mesophilic aerobes and facultative anaerobes tested surfaces (CFU/cm²) after application of both disinfection protocols (mean of three tests)

Tested surface	A	B ^a	R ₁	C	R ₂	Log ₁₀ reduction
<i>Disinfection 1</i>						
Wall	8.83×10^5	1.99×10^3	99.77%	0	100%	3.3
Floor	1.93×10^7	4.38×10^5	97.73%	0.17×10^1	99.9996%	5.41
Ventilation/ heating system	9.37×10^6	5.96×10^4	99.36%	1.05×10^1	99.98%	3.76
Average of all surfaces	9.85×10^6	1.67×10^5	98.30%	0.41×10^1	99.998%	4.61
<i>Disinfection 2</i>						
Wall	5.13×10^5	3.25×10^2	99.94%	0.025×10^1	99.92%	3.11
Floor	1.23×10^7	5.08×10^5	95.87%	0.16×10^1	99.9997%	5.51
Ventilation/ heating system	6.75×10^6	5.01×10^3	99.93%	1.10×10^2	97.8 %	1.66
Average of all surfaces	6.52×10^6	1.71×10^5	97.38%	3.73×10^1	99.98%	3.66

Legend: A – prior to surface cleaning, B – after mechanical surface cleaning, C – after disinfection, R₁ – reduction of microbial contamination after mechanical cleaning (%), R₂ – reduction of microbial contamination after disinfection (%).

Table 2. Number of samples positive for coliforms in both disinfection protocols (10 samples per tested surface)

Tested surface	Before cleaning		After cleaning		After disinfection	
	positive	negative	positive	negative	positive	negative
<i>Disinfection 1</i>						
Wall	10	0	10	0	0	10
Floor	10	0	10	0	0	10
Ventilation/ heating system	10	0	10	0	1	9
Average of all surfaces	30	0	30	0	1	29
<i>Disinfection 2</i>						
Wall	10	0	10	0	0	10
Floor	10	0	10	0	1	9
Ventilation/ heating system	10	0	10	0	6	4
Average of all surfaces	30	0	30	0	7	23

DISCUSSION

The microbial contamination of surfaces of incubators, established in the present study proved the existence of technology flaws and at the same time, confirms that incubators are among the highest source of risk for infection of newly hatched birds.

After the mechanical cleaning, the microbial contamination was significantly reduced. Nevertheless, the microbial counts per unit surface were considerably higher than hygienic allowances. This, however, should not lead to underestimation of the role of mechanical cleaning. As shown by results, it reduced at a great extent the microbial load and allowed the sanitation of the object simultaneously with decreasing the adverse effect of inorganic and organic dirt on the disinfection process. Furthermore, mechanical cleaning is beneficial for the direct contact

of disinfectants and microorganisms, which is a prerequisite of efficient disinfection (Angelov *et al.*, 2006).

After disinfection with 2% Oksisept, the highest extent of microbial reduction was achieved on the incubator wall (100%), and the lowest – on the elements of the ventilation/heating system (99.98%). The residual microflora was in average 4.1 CFU/cm², with lowest viable counts on the wall, followed by the floor and the highest – on ventilation system. The established levels were not higher than the acknowledged critical limits (Samberg & Meroz, 1995; Karadzhov *et al.*, 2004).

Microbiological tests for detection of coliforms demonstrated that they were present in all samples collected before and after the cleaning. After disinfection, there was only one positive sample on the elements of the ventilation system of the incubator – 3.33 % (1/30) of all samples collected after disinfection. According to

Karadzhov *et al.* (2004), the disinfection in livestock facilities is assessed as very good, if the number of samples, positive for coliforms, is no more than 10% of all samples.

On the basis of the present data, the effect of disinfection could be assessed as very good.

The second disinfection protocol, performed with 5% Oksisept as aerosol spray resulted in most significant reduction of microbial load on incubator's flood, whereas the least effect was that on ventilation and heating system. The residual microflora (average for all tested surfaces) was 3.73×10^1 CFU/cm² after disinfection. After application of the second protocol as well, the lowest residual microbial percentage was observed on the wall, followed by the floor and ventilation/heating system elements.

The microbiological tests for coliforms were positive in all samples before and after the cleaning. After disinfection, there was one positive sample from the floor and 6 positive from the ventilation system elements, i.e. 23.33 % (7/30) of all samples were coliform-positive.

The residual microflora and the number of coliform-positive samples obtained from the ventilation system after the disinfection were evaluated as unsatisfactory. The probably reason is their design including numerous details, which, on one hand render difficult the mechanical cleaning and on the other, impede the in-depth penetration of the disinfectant and its regular spread on the surface, especially when applied under the form of a cold mist.

The effect of disinfection on the floor and the walls of the incubator were very good. There was no statistically significant difference in the efficacy of both disinfection protocols tested ($P > 0.05$).

To sum up, the results of disinfections performed allowed concluding that the disinfection efficacy of Oksisept, applied as coarse spray and as cold aerosol spray on incubators in a waterfowl hatchery was high. The high extent of microbial contamination of ventilation system, combined with its more difficult cleaning and disinfection, outlined it as a specific critical point of the incubator, requiring a special attention from the part of disinfection operator.

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